Talanta 160 (2016) 489–498

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Electropolymerized molecular imprinting on glassy carbon electrode for voltammetric detection of dopamine in biological samples

Laszlo Kiss [a,1], Vasile David [b,1], Iulia Gabriela David [b,1], Paul Lazăr [b,1], Constantin Mihailciuc [c,1], Ioan Stamatin [d,1], Adela Ciobanu [e,1], Cristian Dragoş Ştefănescu [f,1], Livia Nagy [a,1], Géza Nagy [a,1], Anton Alexandru Ciucu [b,*,1]

[a] University of Pécs, Szentágothai János Research Center, Pécs, Ifjúság útja 20, 7624 Hungary
[b] University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, 90-92 Panduri Avenue, District 5, Bucharest, 050663 Romania
[c] University of Bucharest, Faculty of Chemistry, Department of Physical Chemistry, 4-12 Regina Elisabeta, District 3, Bucharest, 030018 Romania
[d] University of Bucharest, Faculty of Physics, 3nano-SAE Research Centre, MG38-Magurele, Bucharest, Romania
[e] University of Medicine and Pharmacy, Carol Davila, Department of Psychiatry, Bucharest, Romania
[f] National Institute of Aerospace Medicine, 88 Mircea Vulcanescu Street, Bucharest, Romania

ARTICLE INFO

Article history:
Received 3 February 2015
Received in revised form
5 July 2016
Accepted 7 July 2016
Available online 12 July 2016

Keywords:
Glassy carbon
Polyethacridine
Molecularly imprinted polymer
Dopamine
Voltammetry
Biological samples

ABSTRACT

A simple and reliable method for preparing a selective dopamine (DA) sensor based on a molecularly imprinted polymer of ethacridine was proposed. The molecularly imprinted polymer electrode was prepared through electrodepositing polyethacridine-dopamine film on the glassy carbon electrode and then removing DA from the film via chemical induced elution. The molecular imprinted sensor was tested by cyclic voltammetry as well as by differential pulse voltammetry (DPV) to verify the changes in oxidative currents of DA. In optimized DPV conditions the oxidation peak current was well-proportional to the concentration of DA in the range from 2.0×10^{-8} M up to 1×10^{-6} M. The limit of detection (3σ) of DA was found to be as low as 4.4 nM, by the proposed sensor that could be considered a sensitive marker of DA depletion in Parkinson's disease. Good reproducibility with relative standard deviation of 1.4% and long term stability within two weeks were also observed. The modified sensor was validated for the analysis of DA in deproteinized human serum samples using differential pulse voltammetric technique.

1. Introduction

Dopamine (2-(3,4-dihydroxyphenyl)ethylamine) is one of the most important neurotransmitters which plays a significant role in the central nervous, renal, cardiovascular and hormonal systems [1–3]. Abnormal concentration of dopamine (DA) triggers allergic reactions, schizophrenia or Parkinson disease [4–6] and cardiac arrest [7]. AA is an essential vitamin in the diet of humans; frequently administrated alone or in combination with other drugs in the treatment of the common cold. Additionally, AA may inhibit cytotoxicity induced by oxidants by scavenging peroxyl radicals [8]. UA is the final product of purine metabolism. Kidney stones and gout are associated with an abnormally high level of UA in the body [9]. DA, ascorbic acid (AA) and uric acid (UA) usually coexist together in biological fluids such as blood and urine. Therefore, quantitative determinations of DA, AA and UA have significant implications for biochemical and clinical diagnoses.

A considerable interest has been focused on using simple electrometric methods for the DA measurement in various samples. The mechanism of electrooxidation of DA was intensively investigated by Adams [10]. Since then, intensive efforts were made to measure electroactive neurotransmitters in extracellular liquid of anesthetized animals by employing electrochemical methods like chronoamperometry [11], differential pulse voltammetry [12] or fast scan cyclic voltammetry [13]. For in vivo work, different kinds of small size carbon electrodes have been developed, e.g. a graphite-polyurethane composite electrode [14], a carbon fiber microelectrode [15] or, a carbon fiber electrode coated with overoxidized polypyrrole [16].

There are many difficulties involved in developing electrochemical methods for quantitative analysis of DA such as its tendency to autoxidize [17] and interfering responses from AA and UA [18,19]. In addition, in real biological samples, DA is present in much lower concentration than AA [20]. Sensitivity and selectivity are major properties for the electrochemical sensor. Thus, the development of modified-electrode materials with improved characteristics of selectivity is a challenging task.

* Corresponding author.
E-mail address: anton_ciucu@yahoo.com (A.A. Ciucu).
[1] All authors contributed equally to this work.

http://dx.doi.org/10.1016/j.talanta.2016.07.024
0039-9140/

To achieve selectivity in such measurements, Nagy [21] applied a film of ascorbate oxidase enzyme on the working electrode surface and Gonon [12] performed an electrochemical pretreatment of the carbon fiber electrode. On the same purpose, other scientists modified the carbon paste electrodes with electrocatalysts like iron(II) tetrasulfophthalocyanine [22], nanosized cobalt phthalocyanine particles [23], iron(II) octanitro-phthalocyanine [24], or iron(II)-phthalocyanine [25] and also used the surfactant cetyltrimethylammonium bromide in solutions [23,24] or carbon paste electrode incorporating the surfactant SDS [26,27].

Molecularly imprinted polymers (MIPs) have been recognized as optimal elements to construct sensor with specific binding sites to target molecule [28]. In principle, molecular imprinting techniques can be useful to build up specific host sites on a solid, because of the easy preparation, good selectivity, and robustness in different conditions [29]. A major challenge in developing chemical sensors based on MIPs is to find an effective way of transducing selective recognition process into analytical signal. Sensors based on molecularly imprinted materials, which improve the selectivity of the electrochemical method, for DA detection have been reported [30–37].

Electrochemical polymerization is an attractive procedure among those available for fabrication of the MIP thin films directly on the transducer surface in the presence of the compound to be determined, initially serving as a template [32]. Film thickness is controlled by the amount of charge transferred. Surface morphology is controlled by selection of a suitable solvent and supporting electrolyte. Rigidity and porosity of the film are tuned by solvent swelling and inclusion of ions of a supporting electrolyte. A subsequent removal of the template from the resulting polymer leaves a pattern of molecular vacancies featuring binding sites complementary to those of the template molecules [33,38]. Several transduction schemes, such as those involving optical, electrochemical, or piezoelectric microgravimetry techniques with MIP-aided sensing have already been implemented [28,39–41].

Polymers and oligomers of carbocyclic aryldiamines (phenylenediamines, aminodiphenylamines, etc.) and heterocyclic aryldiamines (diaminopyridines, diaminophenazines, diaminoacridines, and diaminocarbazoles) have received increasing attention during the last two decades [42–44]. Aryldiamines are susceptible to oxidative polymerization via oxidation of one or both amino groups to give linear poly(aminoarylamines), polymers/oligomers containing phenazine units, and ladder polyphenazines. Poly(aryldiamines) have shown tunable electroactivity [45] and high sensibilities of the polymer-modified electrodes to biosubstances at an extremely low concentration [46,47]. Ethacridine lactate (2-ethoxy-6,9-diaminoacridine lactate), is well-known diaminoacridine antiseptic with the trade name Rivanol. As in the case of most polymers and oligomers of aryldiamines, which have been prepared mainly by electrochemical polymerization [42,44], there is one report regarding electropolymerization of ethacridine by potentiostatic and cyclic voltammetric methods [48] at a Pt electrode, however, without any structural characterization of poly (ethacridine) film formed at the electrode surface. Glucose oxidase was simultaneously incorporated into the matrixes of thin poly (ethacridine), which was developed to fabricate a glucose sensor which exhibited good stability and fast amperometric response to glucose [48]. By Raman and FTIR spectra it has been shown that the oxidative polymerization develops free low molecular weight oligomers with specific morphology in plate-like forms, reach in finger print bands. FTIR and Raman spectroscopies also proved the presence of phenazine-like units in ethacridine oligomers [44].

In this paper, the fabrication of a highly selective and sensitive DA sensor was investigated using a molecularly imprinted polyethacridine (MIPET) film as an artificial recognition element electrodeposited on the surface of a glassy carbon electrode (GCE). Dopamine, an important catecholamine neurotransmitter [49], was used as the template molecule in this work because of its prevalence and its electroactivity. To our knowledge, ethacridine has not yet been used in the construction of a MIP sensor for DA detection. Atomic force microscopy, Raman spectroscopy, cyclic voltammetry and differential pulse voltammetry were employed to characterize the constructed sensor. Various performance test results showed that the proposed sensor had good analytical performances such as recognition ability, sensitivity, selectivity and reproducibility toward DA determination. The resulting molecularly imprinted polyethacridine-dopamine-glassy carbon electrode (MIPET-DA-GCE) made possible DA determinations in submicromolar range in the presence of high excesses of electroactive species like ascorbic acid, uric acid and paracetamol.

2. Experimental

2.1. Chemicals, materials

Ethacridine (6,9-diamino-2-ethoxyacridine from Fluka, 98% purity, CAS No. 6402-23-9), in the form of lactate monohydrate ($C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$), was the monomer used in the electrochemical preparation of the polymeric films. 3-hydroxytyramine hydrochloride (dopamine), ascorbic acid, uric acid, and paracetamol, were reagents of analytical purity obtained from Sigma Aldrich. Substances of analytical reagent grade, obtained from Fluka or Sigma-Aldrich, were used to prepare buffers and to process the analyzed blood samples.

Kalium hexacyanoferrat(II) (Merck, Germany) was used to prepare a redox probe solution of $1 \cdot 10^{-2}$ mol L^{-1} $K_4[Fe(CN)_6]$ in phosphate buffer 0.1 M pH = 7.4.

Stock solutions of 1.0×10^{-2} M DA, AA, UA and paracetamol were freshly prepared in 0.1 M phosphate buffer solution (PBS) of pH = 7.4, just before use; DA solutions were kept protected from intensive light, by surrounding the vials with aluminum foil, even when recorded. All solutions were prepared with ultra-pure water.

Deproteinized human serum (DHS) was obtained from the blood samples collected in the morning at clinical hospitals in Bucharest from three different patients and processed as described previously [27].

2.2. Preparation of dopamine-imprinted PET film by cyclic voltammetry

To prepare the MIPET-DA-GCE, a GCE was polished with 1 and 0.05 μm alumina, rinsed with ultra-pure water, and allow to air dry. First, the electrode surface was electrochemically pretreated by performing 10 repetitive cyclic voltammetric scans from −200 mV to 3000 mV vs Ag=AgCl at a scan rate of 500 mV · s^{-1}, in 0.1 M PBS of pH = 7.4, this step ensuring the electrode activation and stabilization and facilitate the polymer adhesion [25]. For the electrodeposition of molecularly imprinted polyethacridine (MIPET), the pretreated GCE was placed into an acetate buffer solution (ABS) of pH = 5.0 containing 10^{-2} M ethacridine lactate (ET) and 10^{-3} M DA and its potential was repetitively cycled between −300 mV and +1500 mV vs Ag=AgCl at a scan rate of 100 mV s^{-1} for five complete voltammetric scans (if not stated otherwise). The pH-value of the electropolymerization solution was identical to that found as optimum in the electrosynthesis of polyethacridine embedded with glucose oxidase [48]. The non-imprinted polymer-modified electrode (NIP) was prepared using the same protocol but in the absence of DA. After electropolymerization of ET (in the presence or absence of DA), the working electrodes were removed from the measuring cell and thoroughly rinsed with ultra-pure water and then air-dried for further characterization.

Please cite this article as: L. Kiss, et al., Talanta (2016), http://dx.doi.org/10.1016/j.talanta.2016.07.024